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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR		ATTORNEY DOCKET NO.
09/058,323	04/09/98	HOUWEN	B	10690/101683

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EXAMINER	
GABEL, G	
ART UNIT	**PAPER NUMBER**
1641	22
DATE MAILED:	09/28/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 09/058,323	Applicant(s) HOUWEN ET AL.	
	Examiner Gailene R. Gabel	Art Unit 1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 August 2001* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-13* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Continued Prosecution Application

1. The request filed on 8/21/01 for a Continued Prosecution Application (CPA) under 37 CFR 1.53(d) based on parent Application No. 09/058,323 is acceptable and a CPA has been established. An action on the CPA follows.

Amendment Entry

2. Applicants' amendment and response filed 8/21/2001 in Paper No. 21 is acknowledged and has been entered. Claim 13 has been amended. Currently, claims 1-13 are pending and under examination.

Rejections Withdrawn

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

3. In light of Applicant's amendment, the rejection of claims 4 and 13 under 35 U.S.C. 112, second paragraph, is hereby withdrawn.

4. In light of Applicant's amendment, the rejection of claims 1-13 under 35 U.S.C. 103(a) as being unpatentable over Loken et al. (US 5,047,321) in view of Kim et al. (US 5,559,037) and Inami et al. (US 5,298,426) is, hereby, withdrawn.

New Grounds of Rejection

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

5. Claims 1-3 and 5-9 rejected under 35 U.S.C. 102(e) as being clearly anticipated by Kim et al. (US 5,648,225).

Kim et al. disclose a method of discriminating and counting erythroblasts using a multipurpose reagent system (see Abstract). The method comprises adding the multipurpose reagent system to an anticoagulated blood sample, incubating the mixture, and subjecting the mixture to flow cytometric analysis (see column 6, lines 6-17). Specifically, the reagent system includes proper concentrations of aldehydes, non-quaternary mono-ammonium salt, and buffer to lyse the nucleated and non-nucleated red cells while maintaining the integrity of the fixed white blood cells (see column 3, lines 60-65). In addition, it includes a buffer that maintains the pH at 5.5-7.5 (see column 7, lines 1-16). Specifically Kim et al. disclose that incubating the blood sample with the reagent system at slightly elevated temperatures, effectively preserves white cell membrane integrity and retains antigenicity of lymphocyte surface antigens (see column 7, lines 37-41). Certain high concentrations of ingredients to lyse nRBC's are

damaging to integrity of white cells and therefore requiring a rapid quenching to the lytic activity of the reagent (see column7, lines 42-47). In addition, the reagent includes a nucleotide fluorescent dye, ethidium homodimer, which reacts with exposed nuclei of nRBCs, i.e. erythroblasts, but impenetrable to intact white cells to allow quantitative analysis of nucleated red cells (see column 8, lines 32-54). The reagent also includes fluorochrome-conjugated antibodies directed to leucocyte surface antigens to allow quantitative analysis and differentiation of leucocytes, i.e. anti-CD4, anti-CD8 conjugated to FITC, PE, etc. (see column 8, line 65 to column 9, line 22). Electronic signals from scattered light collected from different angles and fluorescence intensities are plotted as two dimensional plots (see column 6, lines 31-46 and also Figure 3).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to

consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

6. Claims 4 and 10-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kim et al. (US 5,648,225) in view of Inami et al. (US 5,298,426).

Kim et al. has been discussed supra. Kim et al. differ in failing to lyse, i.e. permeabilize the cell membranes of RBCs and nRBCs by incorporating reagents and buffers at specific pH and osmolality parameters in a two step method such as set forth in claim 4.

Inami et al. disclose a two-step method of differentiating erythroblasts from leucocytes. Inami et al. specifically disclose mixing blood with a hypotonic fluorescent dye solution capable of diffusing into erythroblasts to stain their nuclei and a buffer for maintaining the pH in the acidic range. Inami et al. further mixes the (acidic) sample mixture with a second fluid comprising a buffer that neutralizes the acidic pH in the solution to a staining pH and an osmolarity adjusting agent for adjusting the osmolarity of the solution to a value at which the shape and integrity of leucocytes are maintained (see column 2, lines 3-24 and column 4, lines 17-41). The first acidic and hypotonic fluid has a low osmolality causing erythrocytic cell lines in the sample to swell upon absorbing water causing cellular contents to leak out and nucleotide fluorescent dye (erythroblastic dye to diffuse through the cell membrane to stain their nuclei. Leucocytes do not permit the entrance of nucleotide fluorescent dye (see column 5, line 60 bridging to column 6, line 26). Inami et al. enumerates the different dyes used in the first fluid for differentiating leucocytes and erythroblasts, including propidium iodide and

ethidium bromide specific for erythroblast nuclei, and appropriate concentrations thereof in column 3 of the disclosure. Inami et al. disclose that the concentration of nucleotide fluorescent dye, i.e. propidium iodide or ethidium bromide, should fall within the range of 0.003 mg/L to 10 mg/L (2.5 µg/ml to 100µg/ ml) in order to achieve optimum results (see column 4, lines 5-16). After treatment, stained cells are measured using a flow cytometer and erythroblasts are separated from other cell groups on the resulting two-dimensional plot where erythroblasts are counted (see column 6, lines 9-12). Figure 9 shows a two-dimensional plot showing selective staining of erythroblasts with nucleotide staining dye to emit red fluorescence and to permit erythroblasts to be distributed in a separate zone from other cells so that the relative content and count can be determined. Figure 10 and 11 show two-dimensional plots for the intensity of red fluorescence versus the intensity of side-scattered light obtained for peripheral blood and bone marrow. Inami et al. fail to disclose staining of leucocytes using fluorescent-labeled antibody which specifically binds leucocytes in a hematologic sample.

It would have been obvious to one of ordinary skill in the art at the time of the instant invention to incorporate the teaching of Inami in permeabilizing erythroblasts using reagent combinations having specific pH and osmolality requirements in a two step method, into the reagent system and method disclosed by Kim because Kim specifically taught that integrity and antigenicity of white blood cells need to be maintained optimally during permeabilization, i.e. lysing, of the nRBC's or erythroblasts so as to allow accurate simultaneous quantitation of both populations, sometimes requiring quenching of lytic activity of the reagent because of its damaging effect to

leucocytic populations and Inami specifically taught that such a procedure eliminates such extreme lysing conditions for erythroblasts while maintaining the integrity and shape of WBCs for accurate differentiation of both erythroblast and leucocyte populations.

Kim et al. and Inami are silent in disclosing differentiating between different stages of erythroblast populations. However, Inami specifically disclosed using a same type and concentration of nucleotide fluorescent dye as set forth in claim 10; therefore, it is said that such parameter requirement taught by Inami can effectively perform the same erythroblast maturity differentiation and quantitation as set forth in instant claims 11-12 upon subjecting the sample mixture to flow cytometry .

Response to Arguments

7. Applicant's arguments with respect to claims 1-13 have been considered but are moot in view of the new ground(s) of rejection. Accordingly, no claims are allowed.

Remarks

8. Prior art made of record are not relied upon but considered pertinent to the applicants' disclosure:

Kim et al. (US 5,879,900) disclose a method for simultaneous analysis of cell viability, nRBCs and WBCs.

Terstappen et al. (US 5,057,413) disclose a method foe discriminating between intact and damaged cells.

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gailene R. Gabel whose telephone number is (703) 305-0807. The examiner can normally be reached on Monday to Thursday, 6:30 AM - 4:00 PM and alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached on (703) 308-3399. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 308-4242 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Gailene R. Gabel
September 24, 2001

LONG V. LE
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09/24/01